Applicants wish to thank the Examiner for the corrections which were made to the Sequence Listing for the above-captioned application.

The subject matter newly introduced to claims 1 and 17, which specifies that the vector used in the claimed method comprises an *E. coli* phoA promoter operably linked to a sequence encoding the STII signal peptide, was present in claims 2 and 18 as originally filed. New claims 25 - 30, directed to a method for the efficient production of IFN-α, are supported by page 3, ll. 18-19, Example 5 at page 24, ll. 7-8 and at Table 5, as well as by originally filed claim 9. Thus, no new matter is added by way of this amendment.

In the Office Action mailed May 16, 1996, the Examiner made three rejections to the claims. In response thereto, Applicants respectfully submit the following remarks.

I. The Claimed Invention is Not Obvious Over the Prior Art

A. The Examiner has rejected claims 1-3 and 17-19 under 35 U.S.C. § 103 as being unpatentable over Miyake *et al.* (*J. Biochem.* 97:1429-36 (1985)) in view of Chang *et al.* (U.S. Patent No. 4,963,495) and further in view of Vandlen *et al.* (U.S. Patent No. 5,367,060), Capon *et al.* (U.S. Patent No. 5,455,165), and Baxter *et al.* (U.S. Patent No. 5,258,287).

The Examiner states that:

> [I]t would have been obvious . . . to construct an expression vector for IFN-α according to Miyake, replacing the phoA signal peptide-encoding sequence employed by that reference with the STII signal sequence, thus to make the expression cassette taught by Chang, because Chang teaches that this cassette affords high levels of expression of the heterologous protein product in *E. coli* than does a cassette containing the

> *phoA* signal peptide sequence. . . .Although the prior art clearly does not convey a certainty that the particular combination of this expression cassette with an IFN-α gene would afford high levels of expression, the artisan would have entertained a reasonable expectation of success in successfully practicing such an expression method both because IFN-α was known in the art to be capable of periplasmic secretion in *E. coli*, as evidenced by Miyake, and because the STII leader was generally considered by those skilled in the art to be a suitable signal peptide for the bacterial expression of a wide variety of eukaryotic proteins . . .

Office Action at page 3, lines 11-25.

Applicants traverse this basis for rejection. Applicants submit that the claimed invention is not *prima facie* obvious under 35 U.S.C. § 103 over the cited references for the following reasons.

Applicants respectfully disagree with the Examiner's statement that it would have been obvious to construct an expression vector for IFN-α by replacing the phoA signal peptide-encoding sequence of Miyake *et al.* with the STII signal peptide-encoding sequence, thus making the expression cassette taught by Chang *et al.* Clearly, Miyake *et al.* do not teach or suggest that effective expression of IFN-α could be accomplished by expressing an STII/IFN-α fusion from a phoA promoter. Furthermore, Applicants submit that Chang *et al.* do nothing to cure the deficiencies of Miyake *et al.*

Table 1 of Chang *et al.* (column 5) shows that the amount of human growth hormone (hGH) obtained using a vector comprising a human hGH cDNA ligated to a sequence encoding the STII signal sequence (STII/hGH), under the control of a trp promoter, is twice as high (1 gram) as the amount of hGH produced using a vector comprising STII/hGH under the control of a phoA promoter (0.5 gram). Thus, even if one of ordinary skill in the art were to assume that

the findings of Chang *et al.* regarding hGH expression would be relevant to the expression of IFN-α, the logical construct to make would have been the construct demonstrated to give the highest product yield, *i.e.*, an STII/IFN-α fusion under the control of a trp promoter. Thus, Chang *et al.* teach away from using a construct wherein an STII/IFN-α fusion is expressed from a phoA promoter.

Further, at column 8, ll. 26-34, Chang *et al.* state that "[T]he promoter does not appear to affect the proportion of eukaryotic protein that is secreted. However, it is desirable to screen combinations of promoters and signal sequences for optimal expression since both elements interact in affecting expression levels." However, Applicants have unexpectedly discovered that the choice of promoter is extremely important in obtaining a high product yield of IFN-α. In direct contrast to the findings of Chang *et al.* (that a maximum yield of a mammalian protein (hGH) in *E. coli* is obtained where the protein is expressed as an STII/hGH fusion under the control of a trp promoter), Applicants have found that a much higher yield of IFN-α is obtained when an STII/IFN-α fusion is expressed from a phoA promoter. Applicants direct the attention of the Examiner to the Declaration of Dr. Rudolf Hauptmann, appended hereto (an executed copy of which will be filed as soon as possible). As is explained in this declaration, Applicants unexpectedly found that the IFN-α product yield is three times higher where IFN-α is expressed from the claimed vector construct comprising IFN-α cDNA ligated to a sequence encoding the STII signal sequence under the control of a phoA promoter (phoA/STII/IFN-α) as compared to a STII/IFN-α construct under the control of a trp promoter (trp/STII/IFN-α) (*see* Exhibit B of the Hauptmann declaration). Thus, in direct contravention of the teaching of Chang *et al.*,

Applicants have discovered that a much higher yield of IFN-α is obtained where a phoA/STII/IFN-α construct is used instead of a trp/STII/IFN-α construct.

Further, Applicants submit that Vandlen *et al.*, Capon *et al.*, and Baxter *et al.* do nothing to cure the deficiencies of Miyake *et al.* and Chang *et al.* Although Vandlen *et al.*, Capon *et al.*, and Baxter *et al.* each mention that one possible prokaryotic signal sequence that can be used for expression of mammalian proteins in prokaryotic hosts is the STII signal sequence, it would not have been obvious that the STII signal sequence would be more successful for IFN-α production than any of the other bacterial signal peptides tested, much less that the optimal combination would be the expression of an STII/IFN-α fusion from a phoA promoter.

For the reasons given above, Applicants submit that the claimed invention is not obvious over the cited combination of references, and respectfully request that the rejection under 35 U.S.C. § 103 over Miyake *et al.* in view of Chang *et al.* and further in view of Vandlen *et al.*, Capon *et al*, and Baxter *et al.*, be withdrawn.

B. The Examiner has rejected claims 8, 9, 20, 21 and 24 under 35 U.S.C. § 103 as being unpatentable over Miyake *et al.* in view of Chang *et al.*, Vandlen *et al.*, Capon *et al.*, and Baxter *et al.*, and further in view of Hauptmann *et al.* (U.S. Patent No. 4,917,887). Applicants respectfully traverse this basis for rejection.

The Examiner states that

> [i]t would have been obvious to one of ordinary skill in the art to replace the hIFN-α sequence of Miyake with the hIFN-α2 sequence disclosed by Hauptmann, in a vector incorporating the

> STII signal peptide sequence, as suggested by Chang, in view of Vandlen, Capon, and Baxter, because Hauptmann evidences that the IFN species it encodes was known in the art to be useful.

Applicants respectfully disagree.

For the reasons summarized above, Applicants assert that the use of an expression vector comprising the *pho*A promoter, the STII signal peptide-encoding sequence, and the IFN-α gene would not have been obvious to one of ordinary skill in the art at the time the invention was made. Thus, substitution of the IFN-α2 gene for the IFN-α gene into such an expression vector would also not be obvious. Thus, Applicants assert that the rejection of claims 8, 9, 20, 21 and 24 under 35 U.S.C. § 103 over the cited references has been overcome, and respectfully request that the rejection be withdrawn.

C. The Examiner has rejected claims 4-7 under 35 U.S.C. § 103 as being unpatentable over Miyake *et al.* in view of Chang *et al.*, Vandlen *et al.*, Capon *et al.*, and Baxter *et al.*, and further in view of Protasi *et al.* (U.S. Patent No. 5,066,786) and Higashi *et al.* (U.S. Patent No. 4,828,990).

The Examiner states that

> it would have been obvious to one of ordinary skill in the art at the time the invention was made to purify rhIFN-α produced according to the suggestion of Miyake and Chang as taken in view of Vandlen, Capon, and Baxter, by any methods known in the art for the purification of IFNs, including adsorption on a silicious material as suggested by Protasi, or anion exchange, cation exchange, or hydrophobic interaction (phenyl) chromatography, as suggested by Higashi . . . It would have been *prima facie* obvious to employ any commercial [*sic*] available matrices known to be useful in these procedures, *e.g.*,

silica gel and phenyl-, sulfopropyl-, and DEAE--Sepharose™ or Sephadex™ .

Office Action at page 4, ll. 13-21.

Applicants respectfully traverse this basis for rejection. Applicants submit that as the method of claim 1 is not obvious under 35 U.S.C. § 103, dependent claims 4-7 cannot be deemed obvious merely because they incorporate protein purification steps which may have been known individually in the art. Thus, Applicants submit that claims 4-7 are not obvious under 35 U.S.C. § 103 over the cited references, and respectfully request that the rejection be withdrawn.

II. Conclusion

Applicants respectfully submit that all the bases for objection to the specification and claims, as well as rejection of the claims, have been overcome by the above Amendment and remarks. Reconsideration of the application is respectfully requested, and passage of the application to issuance is earnestly solicited.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

Robert W. Esmond
Attorney for Applicants
Registration No. 32,893

Date: Nov. 18, 1996

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005
(202) 371-2600
P27-26.WPD